

CLAIMS

1. O-glycan α 2,8-sialyltransferase, which is characterized in that it has the following substrate specificity and substrate selectivity.

Substrate specificity: the substrates of the enzyme are glycoconjugates having a Sia α 2,3(6)Gal structure wherein Sia represents sialic acid and Gal represents galactose at the terminus thereof.

Substrate selectivity: the enzyme incorporates sialic acids into O-glycans more preferentially than into glycolipids or N-glycans.

2. O-glycan α 2,8-sialyltransferase having either one of the following amino acid sequences:

- (1) an amino acid sequence shown in SEQ ID NO: 1 or 3; or
- (2) an amino acid sequence comprising a deletion, substitution, and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1 or 3, and having O-glycan α 2,8-sialyltransferase activity.

3. O-glycan α 2,8-sialyltransferase gene encoding the amino acid sequence of the O-glycan α 2,8-sialyltransferase according to claim 2.

4. The O-glycan α 2,8-sialyltransferase gene according to claim 3 which has any one of the following nucleotide sequences:

- (1) a nucleotide sequence corresponding to a portion between nucleotide 77 and nucleotide 1270 of a nucleotide sequence shown in SEQ ID NO: 2;
- (2) a nucleotide sequence comprising a deletion, substitution, and/or addition of one or several nucleotides with respect to the nucleotide sequence corresponding to a portion between nucleotide 77 and nucleotide 1270 of the nucleotide sequence shown in SEQ ID NO: 2, and encoding a protein having O-glycan α 2,8-sialyltransferase activity;
- (3) a nucleotide sequence corresponding to a portion between nucleotide 92 and nucleotide 1285 of a nucleotide sequence shown in SEQ ID NO: 4; and

(4) a nucleotide sequence comprising a deletion, substitution, and/or addition of one or several nucleotides with respect to the nucleotide sequence corresponding to a portion between nucleotide 92 and nucleotide 1285 of the nucleotide sequence shown in SEQ ID NO: 4, and encoding a protein having O-glycan α 2,8-sialyltransferase activity.

5. A recombinant vector comprising the O-glycan α 2,8-sialyltransferase gene according to claim 3 or 4.

6. The recombinant vector according to claim 5 which is an expression vector.

7. A transformant transformed with the recombinant vector according to claim 5 or 6.

8. A method for producing the enzyme according to claim 1 or 2 wherein the transformant of claim 7 is cultured and the enzyme of claim 1 or 2 is collected from the culture.

9. A protein which comprises an active domain of O-glycan α 2,8-sialyltransferase having any one of the following amino acid sequences:

(1) an amino acid sequence corresponding to a portion between positions 26 and 398 of the amino acid sequence shown in SEQ ID NO: 1;

(2) an amino acid sequence comprising a deletion, substitution, and/or addition of one or several amino acids with respect to the amino acid sequence corresponding to a portion between positions 26 and 398 of the amino acid sequence shown in SEQ ID NO: 1, and having O-glycan α 2,8-sialyltransferase activity;

(3) an amino acid sequence corresponding to a portion between positions 68 and 398 of the amino acid sequence shown in SEQ ID NO: 3; and

(4) an amino acid sequence comprising a deletion, substitution, and/or addition of one or several amino acids with respect to the amino acid sequence corresponding to a portion between positions 68 and 398 of the amino acid sequence shown in SEQ ID NO: 3, and having O-glycan α 2,8-sialyltransferase activity.

10. An extracellular secretory protein, which comprises a polypeptide portion which is an active domain of the O-glycan α 2,8-sialyltransferase of claim 1 or 2, and a signal peptide, and has O-glycan α 2,8-sialyltransferase activity.

11. A gene encoding the protein according to claim 9 or 10.

12. A recombinant vector comprising the gene according to claim 11.

13. The recombinant vector according to claim 12 which is an expression vector.

14. A transformant transformed with the recombinant vector according to claim 12 or 13.

15. A method for producing the protein according to claim 9 or 10 wherein the transformant of claim 14 is cultured and the protein of claim 9 or 10 is collected from the culture.

16. β -galactoside α 2,6-sialyltransferase, which is characterized in that it has the following action and substrate specificity.

(1) Action;

The enzyme transfers sialic acid through an α 2,6 linkage into the galactose portion of a sugar chain having a galactose β 1,4N-acetylglucosamine structure at the terminus thereof.

(2) Substrate specificity

The substrate of the enzyme is a sugar chain having a galactose β 1,4N-acetylglucosamine structure at the terminus thereof, and lactose and a sugar chain having a galactose β 1,3N-acetylglucosamine structure at the terminus thereof are not the substrate of the enzyme.

17. β -galactoside α 2,6-sialyltransferase having either one of the following amino acids:

(1) an amino acid sequence shown in SEQ ID NO: 5 or 7; or

(2) an amino acid sequence comprising a deletion, substitution, and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 5 or 7, and having β -galactoside α 2,6-sialyltransferase activity.

18. A β -galactoside α 2,6-sialyltransferase gene encoding the amino acid sequence of the β -galactoside α 2,6-sialyltransferase according to claim 17.

19. The β -galactoside α 2,6-sialyltransferase gene according to claim 18 which has any one of the following nucleotide sequences:

(1) a nucleotide sequence corresponding to a portion between nucleotide 176 and nucleotide 1762 of a nucleotide sequence shown in SEQ ID NO: 6;

(2) a nucleotide sequence comprising a deletion, substitution, and/or addition of one or several nucleotides with respect to the nucleotide sequence corresponding to a portion between nucleotide 176 and nucleotide 1762 of the nucleotide sequence shown in SEQ ID NO: 6, and encoding a protein having β -galactoside α 2,6-sialyltransferase activity;

(3) a nucleotide sequence corresponding to a portion between nucleotide 3 and nucleotide 1574 of a nucleotide sequence shown in SEQ ID NO: 8; and

(4) a nucleotide sequence comprising a deletion, substitution, and/or addition of one or several nucleotides with respect to the nucleotide sequence corresponding to a portion between nucleotide 3 and nucleotide 1574 of the nucleotide sequence shown in SEQ ID NO: 8, and encoding a protein having β -galactoside α 2,6-sialyltransferase activity.

20. A recombinant vector comprising the β -galactoside α 2,6-sialyltransferase gene according to claim 18 or 19.

21. The recombinant vector according to claim 20 which is an expression vector.

22. A transformant transformed with the recombinant vector according to claim 20 or 21.

23. A method for producing the enzyme according to claim 16 or 17, wherein the transformant of claim 22 is cultured and the enzyme of claim 16 or 17 is collected from the culture.

24. A protein comprising an active domain of β -galactoside α 2,6-sialyltransferase having any one of the following amino acid sequences:

- (1) an amino acid sequence corresponding to a portion between positions 33 and 529 of the amino acid sequence shown in SEQ ID NO: 5;
- (2) an amino acid sequence comprising a deletion, substitution, and/or addition of one or several amino acids with respect to the amino acid sequence corresponding to a portion between positions 33 and 529 of the amino acid sequence shown in SEQ ID NO: 5, and having β -galactoside α 2,6-sialyltransferase activity;
- (3) an amino acid sequence corresponding to a portion between positions 31 and 524 of the amino acid sequence shown in SEQ ID NO: 7; and
- (4) an amino acid sequence comprising a deletion, substitution, and/or addition of one or several amino acids with respect to the amino acid sequence corresponding to a portion between positions 31 and 524 of the amino acid sequence shown in SEQ ID NO: 7, and having β -galactoside α 2,6-sialyltransferase activity.

25. An extracellular secretory protein, which comprises a polypeptide portion which is an active domain of the β -galactoside α 2,6-sialyltransferase according to claim 16 or 17, and a signal peptide, and has β -galactoside α 2,6-sialyltransferase activity.

26. A gene encoding the protein according to claim 24 or 25.

27. A recombinant vector comprising the gene according to claim 26.

28. The recombinant vector according to claim 27 which is an expression vector.

29. A transformant transformed with the recombinant vector according to claim 27 or 28.

30. A method for producing the protein according to claim 24 or 25 wherein the transformant of claim 29 is cultured and the protein of claim 24 or 25 is collected from the culture.